

Transformation

Transformation of electrocompetent cells

- 50 µL aliquot of electro competent *E. coli* cells is thawed on ice
- Pre-chill plasmid DNA and electroporation cuvettes on ice
- Add 50 ng of plasmid DNA to the cells
- Transfer mixture to the electroporation cuvette (0.1 cm)
- Electroporation is performed in the electroporator by applying 1.8 kV
- Add 950 µL LB medium to transformed cells immediately
- Transfer suspension to a sterile 1.5 mL microfuge tube
- For cell recovery the culture is incubated at 37 °C and 4500 rpm for 30 min – 1 h.
- Plate cells on medium-agar plates with appropriate supplements i.e. antibiotics and incubate overnight at 37°C

Transformation of CaCl₂ competent *E. coli* cells

- 50 µL or 100 µL aliquot of chemically competent *E. coli* cells is thawed on ice
- Add 50 – 100 ng of plasmid DNA to cells
- Incubate mixture on ice for 20 minutes followed by a heat shock at 42 °C for 90 sec and again incubate on ice for 2 minutes
- Add 1 mL LB medium immediately
- recover cells at 37 °C and 450 rpm for 30 min - 1 h
- plate cells on agar plates with appropriate supplements i.e. antibiotics and incubate overnight at 37 °C